Effect of Salt on Bacterial Denitrification of Agricultural Drainage Water

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Abstract The Algal-Bacterial Selenium Removal (ABSR) system has demonstrated an ability to efficiently remove nitrogen from agricultural drainage water in the San Joaquin Valley. However, it is not known whether the ABSR system can effectively reduce nitrate and nitrite concentrations in drainage water with high levels of salinity. In order to determine whether the ABSR system can be used to treat salty drainage water in the future, the effectiveness of nitrogen removal from drainage water with high salt concentrations must be examined. Two laboratory experiments were conducted to study the effect of salt on bacterial denitrification of drainage water. The first experiment involved evaporating and diluting typical drainage water into bottles with different salt concentrations and equal amounts of molasses and bacteria. Dissolved oxygen, pH, and turbidity were monitored and the bottles were periodically tested for nitrate and nitrite concentrations. Similar procedures were used for the second experiment, but four different salt concentrations were achieved through the addition of salt. Denitrification was slower at higher salt concentrations for the first experiment, however, nitrogen removal may have been affected by higher nitrate concentrations at higher salinity. The results from the second experiment showed that bacterial denitrification occurred at the same rate for all four different salinities, and that salt had no significant effect on nitrogen removal. The results from this study suggest that salt does not hinder bacterial denitrification of drainage water, and that the ABSR system can be used to remove nitrogen from agricultural drainage water with salt concentrations of up to 22 g/L. Future research can help determine how other factors may affect bacterial denitrification of agricultural drainage water.

Introduction

The San Joaquin Valley in California is one of the most productive agricultural regions in the world. However, agriculture in the San Joaquin Valley relies on heavy crop irrigation because of low annual precipitation in the dry region. Subsurface drainage systems are constructed beneath agricultural fields in the valley to transport excess drainage water and runoff to a main canal (Botkin and Keller 2000). The drainage of agricultural wastewater has been a problem in the San Joaquin Valley since the 1950's, when agricultural activities increased dramatically. A tiny percentage of salt minerals are present in freshwater resources, however, salt is a problem in the Central Valley because of heavy irrigation and over pumping of groundwater for farming. Salt minerals accumulate in soils and groundwater over time because water used in agricultural irrigation evaporates quickly and salt is left behind. The build up of salt from evapotranspiration is further compounded because more and more salty water is pumped from underground and used for irrigation. As a result, large amounts of salt are accumulating in the San Joaquin Valley. According to the California Department of Water Resources, salt is accumulating at a rate of 2.45 million tons per year in the Central Valley (Follette 2002).

The buildup of salt in soil and groundwater is problematic because many agricultural crops cannot tolerate salty water and some aquatic organisms cannot survive in high saline water (Meng and Moyle 1995). In response to the drainage problem in the San Joaquin Valley, the San Luis Drain project was initiated in 1968 to construct a long irrigation drainage canal from the San Joaquin Valley to the Pacific Ocean (Botkin and Keller 2000). The drain would have carried salty drainage water from the San Joaquin Valley and disposed it into the San Francisco Bay. However, the San Luis Drain project was never completed due to budget cutbacks and environmental concerns from the Bay Area community. Instead, the 132-kilometer long partially completed drain stopped at Kesterson Reservoir and agricultural wastewater was dumped there. In 1983, it was discovered that the occurrence of deformed and dead migratory birds at Kesterson Reservoir was due to high levels of selenium in the water (Ohlendorf et al. 1986). The San Luis drain and the Kesterson Reservoir were closed in 1985 due to concerns over the threat of toxic selenium on wildlife and public health (Botkin and Keller 2000).

Ever since the closure of Kesterson Reservoir in 1985, there have been many attempts by the government to manage the drainage problem in the Central Valley and to deal with the selenium in agricultural wastewater. Agricultural waste water from the Central Valley cannot be exported

or disposed of elsewhere without first removing the toxic selenium in the water. One of the ways that selenium can be efficiently removed from drainage water at low cost is by the Algal-Bacterial Selenium Removal (ABSR) system. The ABSR system, designed by UC Berkeley professor William Oswald, consists of two artificial ponds that utilize bacteria and algae to remove nitrate and selenium from agricultural drainage water (LBNL 2000). Bacterial denitrification is an important process in the ABSR system. The presence of nitrogen hinders selenium removal from drainage water because bacteria prefer reducing nitrate and nitrite before converting selenate to the more easily removable selenite and elemental selenium (Lee 2002). Therefore, nitrogen must be removed before the removal of selenium can successfully occur. Past research studies have shown that the ABSR system can effectively remove nitrogen and selenium from drainage water in the Central Valley. A pilot ABSR facility constructed near Los Banos in the Central Valley has demonstrated an ability to remove 95% of the influent nitrogen and 80% of the influent selenium (LBNL 2000).

Although the ABSR system can effectively remove selenium and nitrogen in typical drainage water with salt concentration ranging from 6 to 8 g/L, the ability of the ABSR system to remove selenium and nitrogen from high saline water is unknown. One of the ways to remove salt from drainage water is through reverse osmosis, also known as hyperfilitration. Many reverse osmosis plants have been built in the Central Valley to help remove salt minerals from agricultural drainage water. However, reverse osmosis leaves behind concentrated impurities that are high in salt and contain high levels of selenium. Toxic impurities leftover from reverse osmosis is a danger to wildlife and human health if the selenium in the impurities is not removed. Therefore, it is critical that a mechanism be developed to treat highly concentrated salt water and remove toxic selenium.

In the future, the ABSR system might be used to treat impurities from reverse osmosis plants or treat drainage water that have higher salt concentrations (Lundquist 2002 pers. comm.). Currently, the ABSR ponds in Panoche treat agricultural drainage water that has about 6 to 8 g/L of total dissolved solids. However, the reverse osmosis plants leave behind salt concentrated impurities that contain 12 to 80 g/L of total dissolved solids (Lundquist 2002 pers. Comm.). Studying nitrogen removal in the ABSR system is important because nitrate and nitrite removal is critical to the removal of selenium in the ABSR system. Studying denitrification and nitrogen removal in agricultural wastewater is also important for many reasons (Follett and Hatfield

2001), such as eutrophication of lakes (Welch 1992), public health (Fan and Steinberg 1996), and groundwater purity (Kross et al. 1993).

The objective of my research project was to help determine whether the ABSR system can be used to treat salty drainage water by examining the process of bacterial denitrification at high salt concentrations. Build an actual ABSR facility is expensive, therefore, experiments must be conducted beforehand to determine if the ABSR system can potentially be used to treat drainage water with high salt concentrations. My research project consisted of designing two experiments to determine the effectiveness of nitrogen removal at different salinity. I hypothesize that denitrification of drainage water will occur at different salinities, but nitrogen removal will be affected by salt. Nitrate and nitrite removal efficiency will decrease at higher salt concentrations because bacteria and algae will not be able to thrive under high saline environment.

Methods

Two laboratory experiments were conducted at Lawrence Berkeley National Laboratory (LBNL). The first experiment, conducted from September 15th to October 17th, lasted for 31 days. The second experiment, from November 1st to the 15th, lasted for 14 days. Agricultural drainage water was collected from the ABSR facility near Los Banos in the San Joaquin Valley on September 6, 2002 and brought back to LBNL. The drainage water, hereafter referred to as PI water, is the Panoche influent from the Panoche drainage district. On September 11, 2002, 250 ml of water sample was taken from the north reduction (RN) pond at the ABSR facility. The water collected from the north reduction pond, abbreviated as RN water, contains bacteria used to remove nitrate. 2,500 ml of PI water was allowed to evaporate in an oven at 240°C for about seven hours. After evaporation, the concentrated PI solution was diluted with deionized water to give three beakers that each contained 530 ml of water with salt concentrations of 100% (1x), 150% (1.5x), or 200% (2x) of the original concentration of the PI water.

The three beakers with different salt concentrations each received equal amounts of bacteria and molasses. Five ml of bacterial seed solution that contain bacteria and algae that have been living in a high salinity environment since April 2001 was added to each of the three beakers. Five ml of RN water collected on September 11th was added into each of the three beakers. 18.6

grams of pure molasses were mixed with 100 ml of water in an erlenmeyer flask, and 1 ml of the mixed molasses solution was added into each of the three beakers. RN water and molasses (as a food resource) was added to the beaker to help the bacteria maximize their potential for growth in a new environment (Lundquist 2002 personal comm.). After mixing the beakers thoroughly, each of the three beakers was divided into six 100-ml bottles with 65 ml of solution in each bottle. Three 65 ml bottles of unaltered Panoche drainage water were also prepared and labeled as PI water The three PI bottles also received the same amount of bacteria and molasses as the other bottles. As a result, there were six bottles of 1x, 1.5x, and 2x, plus three bottles of PI for a total of 21 bottles.

After the addition of bacteria and molasses into each of the 21 bottles, helium gas was used to remove dissolved oxygen that is present in the solution by a technique called sparging. Each of the bottles was sparged with helium gas for two and half minutes at 30 psi. The sparge tube was moved in a circular motion to make sure there is equal sparging in the bottle and to remove as much dissolved oxygen as possible in the solution (Brent 1997). After the bottles were sparged, the bottles were transferred into an anaerobic chamber (Bactron Anaerobic Chambers, Sheldon Manufacturing Inc). The anaerobic chamber, commonly referred to as a glove box, is used to drive out the remaining oxygen in the bottles and to seal the bottles in an anaerobic environment. After all 21 bottles were sealed; the bottles were put in a shallow bucket and stored in an incubator at 35°C to help increase bacterial and algae growth.

During the duration of the experiment, all 21 bottles were vigorously shaken thirty times in a uniform direction once a day. Bottles needed to be shaken to prevent bacterial from settling to the bottom and sticking to the bottles. On day 0, 3, 6, 13, 18 and 31 of the experiment, one bottle of each concentration (1x, 1.5x, 2x) was opened and sampled. The PI bottles were sampled on day 0, 6, and 18. Each of the opened bottles was measured for pH, dissolved oxygen, and turbidity. The pH was measured using the Fisher pH meter, dissolved oxygen was measured using the Orion O2 probe, and turbidity was measured with a turbidity meter. Afterwards, 40 ml from each bottle were filtered through a 0.22-micron filter and stored in two 25-ml vials. One vial would be a sample that is filtered and acidified, and the other vial would be a sample that is filtered but not acidified. The acidified sample would be used to test for nitrate (NO3) concentrations and the unacidified sample would be tested for nitrite (NO2) concentrations. The

filtered and unacidified must be tested within 48 hours because nitrite would turn into nitrate after about two days (APHA 1995). Standard methods of nitrite and nitrate analysis were used (Lee 2002, Kurosu 2001) with the exception that 250 ul of sample was injected into each of the three well, instead of 300 ul.

The second experiment was conducted following the completion of the first experiment. The methods used for the second experiment were similar to the first experiment, however, there were some major differences. For the second experiment, instead of using evaporation and dilution, drainage water with four different salt concentrations were achieved through the addition of sodium chloride (NaCl). The salt concentration of the original PI drainage water was determined from total dissolved solids (TDS) measurements, and the amount of NaCl needed to achieve the desired salt concentrations were calculated. 540 ml of the unaltered PI water with no salt added, labeled as 1x, would serve as drainage water with 100% salt concentration. To get drainage water with salinity of 150% (1.5x) of PI drainage water, 2.255 g of NaCL was added to 540 ml of PI water. To get salt concentration of 200% (2x), 4.955 g of NaCl was added to 540 ml of PI water. For 250% (2.5x), 7.115 g of NaCl was added to 540 ml of PI water.

For the second experiment, seven bottles of each salt concentration (1x, 1.5x, 2x, and 2.5x) were prepared, for a total of 28 bottles. Like the first experiment, each 100-ml bottle contained 65 ml of solution and each bottle received equal amounts of molasses and bacteria. However, different bacteria were used in the second experiment. The bacteria used in the second experiment were taken from a high salinity drainage field in the Central Valley called Red Rock Ranch. The bacteria from Red Rock Ranch were supposed to be more salt tolerant than the bacteria used in the first brines experiment because the they have been living in a high salinity environment (Lundquist 2002 pers. comm.). To save time and increase the rate of bacterial denitrification, more bacteria were added to each bottle for the second experiment. It is estimated that each bottle received about 1 mg/L of bacteria from Red Rock Ranch (Lundquist 2002 pers. comm.). To drive out the remaining dissolved oxygen in the solution, each bottle was shaken for 30 seconds in the glove and sealed in an anaerobic environment. The 28 bottles were placed in an automatic shaker and stored in an incubator at 35°C.

During the second experiment, one bottle of each salt concentration (1x, 1.5x, 2x, and 2.5x) was opened on day 0, 3, 6, 9 and sampled. On the last day of the experiment, day 14, three

bottles of each concentration were opened and tested. Following procedures described for the first experiment, samples from each bottle were filtered and tested for nitrate and nitrite concentrations, and measurements of pH, dissolved oxygen, and turbidity were taken.

The results of the first and second experiment were entered in a Microsoft Excel spreadsheet and the progression of nitrate and nitrite concentrations over time were graphed and examined. Total nitrate and nitrite, nitrite concentration, dissolved oxygen, and turbidity measurements were plotted against each other and tested for correlation. Day 14 results from the second experiment were used for the Tukey Honestly Significant Difference (HSD) test. The Tukey HSD test was used to examine for differences in total NO3 and NO2, NO2, turbidity, and dissolved oxygen due to differences in salt concentrations.

Results

Nitrogen removal occurred at all three different salt concentrations for the first experiment. The progression of bacterial denitrification over time can be observed in Figure 1 and Figure 2. For the first experiment, nitrate concentrations started at 39.4 mg/L for PI, 39.9 mg/L for 1x, 55

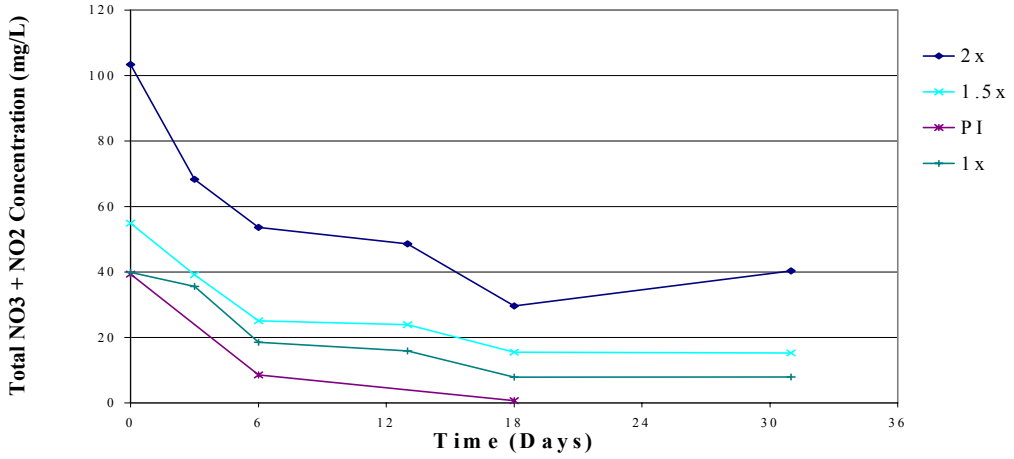

Figure 1. Total NO3 + NO2 during first experiment.

mg/L for 1.5x, and 103.4 mg/L for 1x. After 31 days, drainage water at 100% (1x) salt concentration had about 7.5 mg/L of total nitrate and nitrite remaining. The total nitrate and nitrite concentration was about 15.3 mg/L for 150% (1.5x), and 40 mg/L for 200% (2x) salt concentration. PI water, the unaltered drainage water that served as control, experienced complete denitrification and had zero nitrite concentration after 18 days (Figure 2).

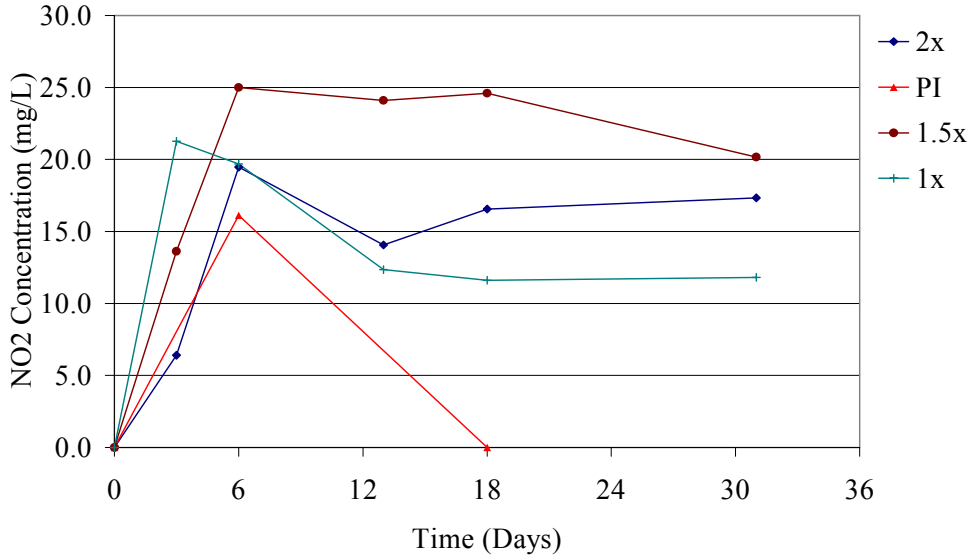

Figure 2. NO2 concentration during first experiment.

During the first experiment, denitrification stagnated from day 18 to day 31. Not much nitrogen removal occurred after day 18. Nitrate concentrations and nitrite concentrations were tested for correlation with dissolved oxygen and turbidity. No correlation was found between nitrogen concentration and levels of dissolved oxygen, and there was no significant correlation between nitrogen removal and turbidity.

For the second experiment, bacterial denitrification was successful at all four different salt concentrations. Almost all nitrate and nitrite were removed from drainage water after 14 days (Figure 3). Less than 1 mg/L of nitrate and nitrite remained after day 14 of the experiment. Figure 4 describes the progression of NO2 concentration over time. Nitrite concentrations rose sharply on day three following the conversion of nitrate to nitrite. Nitrite concentrations

decreased over time from day three until the end of the experiment on day 14. On day 14, nitrite concentrations were essentially zero for salt concentrations of 1.5x, 2x, 2.5x, while there were

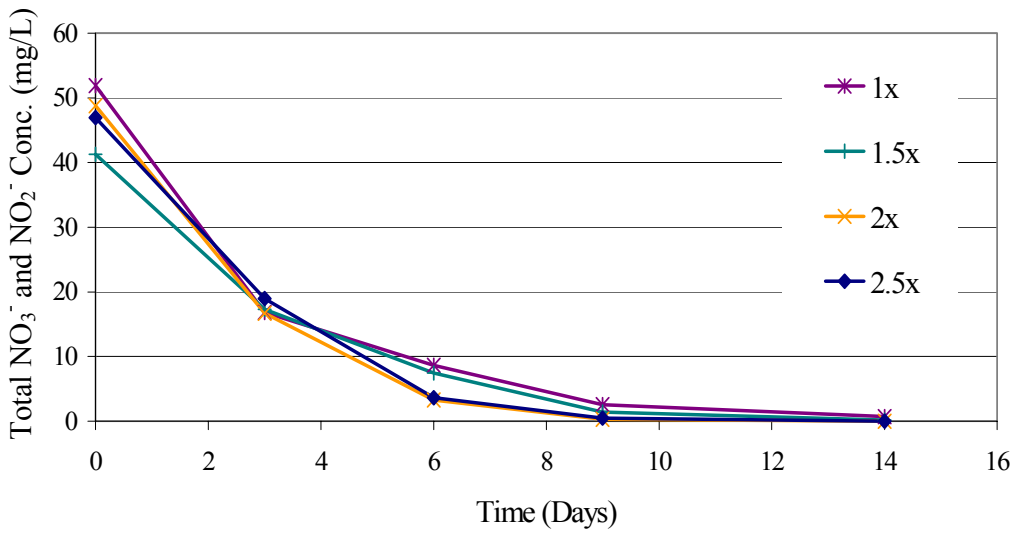

Figure 3. Total NO3 and NO2 concentrations during second experiment.

still 2 mg/L of nitrite left in 1x drainage water (Figure 4).

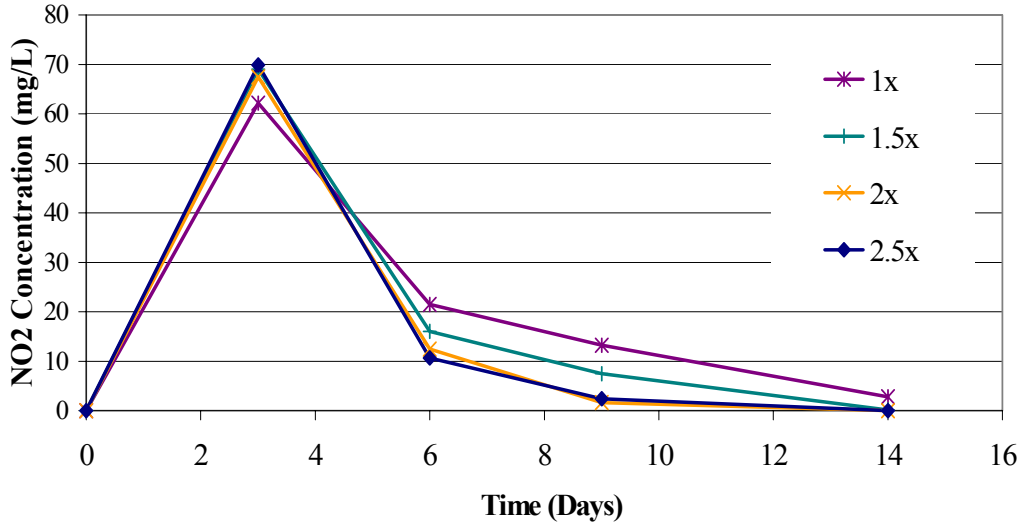

Figure 4. NO2 concentration during second experiment.

The Tukey Honestly Significant Difference (HSD) test was used to test for whether the different nitrite concentrations on day 14 of the second experiment were due to different

salinities. The Tukey HSD test showed that the differences in nitrite concentration on day 14 were not significantly related to the different salt concentrations, at 95% confidence. The Tukey HSD test also showed that differences in total NO3 and NO2, dissolved oxygen, and turbidity measurements were not due to salt, at 95% confidence level. In other words, we can be 95% confident that nitrate and nitrite concentrations, dissolved oxygen, and turbidity did not differ significantly between the four different salt concentration in experiment two.

Discussion

The results from the first experiment showed that drainage water with higher salt concentrations ended up with higher concentrations of nitrate and nitrite on day 31. This would seem to suggest that salinity was a hindrance to nitrogen removal in the experiment. However, careful examination of the data and the graph in Figure 1 reveals that each salt concentration had different nitrogen concentration at the start of the experiment. At higher salinity, the nitrate concentrations were higher. For example, at 2x or 200% of the salt concentration of typical drainage water, the nitrate concentration was 103 mg/L, compared to PI water or 1x, which had nitrate concentrations around 40 mg/L. The different rates of denitrification at different salt concentrations in experiment one could have been due to salt or denitrification could have been slowed by the high initial nitrogen concentration in the water. The results from the second experiment helped us better understand the results for experiment one. The results from experiment two suggest that salt have no significant effect on nitrogen removal at higher salt concentrations. This means that higher nitrate and nitrite concentration by the end of the first experiment on day 31 was not due to the effect of salt, but rather due to high initial nitrate concentrations.

For the second experiment, four different salt concentrations were achieved through the addition of NaCl, but initial nitrate concentrations were supposed to be the same for all salinities. In fact, the initial nitrate concentrations on day 0 ranged from 41 mg/L to 52 mg/L. However, the initial difference between nitrate concentration can largely be attributed to natural variations that occur when nitrate concentration is measured and possible human errors in measurement. During the second experiment, bacterial denitrification occurred at similar rates for all four salt concentrations. By the end of day 14, almost all nitrogen was removed from the drainage water

at different level of salinity as total nitrate and nitrite concentration all approached zero. The curve in Figure 3 and Figure 4 suggest that all four salt concentrations had very similar nitrate and nitrite progression trends over time. The Tukey HSD test provided further evidence that different salt concentration had little effect on bacterial denitrification in experiment two.

The results from my experiment suggest that salt has no effect on nitrogen removal and that bacterial denitrification can still be effective at high salinity. The implications from this study suggest that the ABSR system can be used to treat high salty drainage water and salt does not hinder bacterial denitrifiication. However, we can only be confident that nitrogen removal by bacteria will not be hindered by salt at salt concentrations of up to 22 g/L, which was the salinity of 2.5x in the second experiment. We are still not sure of the exact role that salt plays in bacterial denitrification. For instance, nitrogen removal may well be hindered by salt at salt concentrations over 50 g/L. Continual research is necessary to test bacterial denitrification at even higher salt concentrations, such as 35 g/L (4x) or 44 g/L (5x). Besides testing nitrogen removal at higher salt concentrations, future research should also try to test bacterial denitrification on high salty water that has proportionally high levels of nitrate. In the San Joaquin Valley and other places in the world, high salty drainage water also has high levels of nitrate. From experiment one, we learned that although nitrogen removal happened at all three different salt concentrations, the relative rate of denitrification is probably affected by the initial concentration of nitrate in the water. Therefore, high levels of nitrate in water may ultimately be a big problem for bacterial denitrification by the ABSR system. The efficiency of denitrification has importance implication when hydraulic retention time in the ABSR system is considered. If nitrogen cannot be efficiently removed, selenium removal will not be successful in the ABSR system (LBNL 2000). Future research should try to better understand the limitation of the bacterial denitrification process and figure out the best method to treat high salty drainage water with high levels of nitrate and selenium.

Besides considering salinity and levels of nitrogen, other factors such as dissolved oxygen need to be taken into account when studying bacterial denitrification. Previous researches have shown that dissolved oxygen is a hindrance to nitrogen removal because bacteria prefer using oxygen before reducing nitrate to nitrite (Lee 2002). Although there was no significant correlation between nitrogen removal and levels of dissolved oxygen at 95% confidence, it is possible that oxygen may still have affected nitrogen removal efficiency in the experiment. The

dissolved oxygen measurements in the two experiments were relatively high and indicated the presence of dissolved oxygen in the bottles. Bacterial denitrification was supposed to take place in an anoxic environment, but oxygen may have leaked into the bottles through the rubber cap over time since the bottles were stored in an oxygen environment, and affected bacterial denitrification. An anoxic environment is desirable for bacterial denitrification because the bacteria will use the oxygen in the water before they will reduce nitrate to nitrite. Dissolved oxygen was a concern in the experiment because the Orion O2 probe did not accurately measure dissolved oxygen in the bottles (Lundquist 2002 pers. comm.). There was a wide range in the measurements of dissolved oxygen and the level of dissolved oxygen could not be measured accurately. Future experiments should try to prevent any dissolved oxygen in the bottles and the levels of dissolved oxygen should be monitored correctly.

Molasses plays an important role in the bacterial denitrification of agricultural drainage water. Previous studies have shown that molasses greatly increase nitrogen removal in the north reduction pond at the ABSR facility because bacteria are able to use molasses as a food resource (Lee 2002). A nitrogen removal experiment conducted by the Tryg Lundquist and his applied algae research group also showed that denitrification is enhanced by adding molasses (Brent 1997). During the first experiment, there was very little nitrogen removal from day 18 to day 31. The stagnation in denitrification and the lack of nitrogen removal after day 18 could be explained by molasses availability. The threshold for nitrogen removal may have been reached after day 18 because molasses as a food resource for bacteria may have been used up by after day 18. Bacteria may have been unable to reduce any more nitrite because molasses in the bottles ran out. While we do not know why denitrification stagnated after day 18, the importance of molasses as a food resource for bacteria in bacterial denitrification is worth considering when designing future experiments.

This study gave us a better understanding of the effect of salt on bacterial denitrification of agricultural drainage water and increased our knowledge on the all complications involved with using bacteria to remove nitrogen from water. Hopefully, the results from my research project will help pave the way for more research in the future on how to effectively remove selenium and nitrate from high saline agricultural drainage water in the San Joaquin Valley. Although my experiments demonstrated that the ABSR system can potentially be used to treat salty drainage

water and remove nitrogen, more research and careful planning is needed before a real ABSR system can be built and used to treat salty drainage water. Nevertheless, the results from my study have important implications for future water treatment research. The data from my research suggest that bacterial denitrification can effectively remove nitrate and nitrite from drainage water with salt concentrations ranging from 8.8 to 22 g/L. If the ABSR system can indeed be used to treat high salty drainage water, then the drainage problem that have plagued the San Joaquin Valley for the past fifty years may finally be managed effectively.

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References

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. , 16th ed., A.E. Greenberg, R.R. Rhodes Trussell, L.S. Clesceri, eds, American Public Health Association, American Water Works Association, and Water Environment Federation.

Botkin, D.B., and E.A. Keller. 2000. Environmental science: Earth as a living planet. John Wiley & Sons, New York. 664pp.

Brent, A. 1997. Nitrate Removal Batch Test. Unpublished report for the applied algae research group at UC Berkeley.

Fan, A.M., and V.E. Steinberg. 1996. Health implications of nitrate and nitrite in drinking water: an update on methemoglobinemia occurrence and reproductive and developmental toxicity. Regulatory Toxicology and Pharmacology 23: 35-43.

Follett, R.F, and J.L. Hatfield. 2001. Nitrogen in the Environment: sources, problems, and management. Elsevier Science, New York. 520 pp.

Follette, D. 2002. Salt-Balance in California's San Joaquin Valley. LBNL website. http://www.lbl.gov/Education/CSEE/cup/Su01/Sum01Web/Follette/wqm.html accessed September 14, 2002.

Kross, B.C., G.R. Hallberg, D.R. Bruner, K. Cherryholmes, and J.K. Johnson. 1993. The nitrate contamination of private well water in Iowa. American Journal of Public Health 83: 270-272.

Kurosu, O. 2001. Nitrogen removal from wastewater in Microalgal-bacterial treatment ponds. Investigating the Environment: Research for Environmental Management.

Lawrence Berkeley National Laboratory (LBNL). 2000. The Algal-Bacterial Selenium Removal Facility at the Panoche Drainage District. Drainage Treatment Bulletin, October 2000.

Lee, J. 2002. Nitrogen mass balance of the ABSR system and comparison of nitrate removal of its two modes. Investigating the Environment: Research for Environmental Management.

Lundquist, Tryg. Associate Specialist, Lawrence Berkeley National Laboratory, Berkeley, CA. 2002, personal communication.

Ohlendorf, H.M. et al. 1986. Embryonic mortality and abnormalities of aquatic bird: apparent impacts of selenium from irrigation drainage water. The Science of the Total Environment: 52: 49-63.

Meng, L., and P.B. Moyle. 1995. Status of splittail in the Sacramento-San Joaquin estuary. Transactions of the American Fisheries Society 124: 538-549.

Welch, E.B. 1992. Ecological effects of wastewater: applied limnology and pollutant effects. Chapman & Hall, New York. 456 pp.

Appendix

First Experiment Data							
		Day 0	Day 3	Day 6	Day 13	Day 18	Day 31
PI	Dissolved Oxygen (mg/L)	N/A		0.85		0.1	
	Turbidity(NTU)	N/A		34.45		13.55	
1x	Dissolved Oxygen	N/A	0.25	0.25	0.9	N/A	0.9
	Turbidity(NTU)	N/A	17.5	14.65	13.1	12.9	10.6
2x	Dissolved Oxygen (mg/L)	N/A	0.25	0.55	0.67	N/A	0.93
	Turbidity (NTU)	N/A	17.6	14.25	11.8	11.7	9.85
2.5x	Dissolved Oxygen (mg/L)	N/A	0.45	0.375	0.96	N/A	0.88
	Turbidity (NTU)	N/A	19.05	15.25	14	12.05	10.95

Second Experiment Data						
		Day 0	Day 3	Day 6	Day 9	Day 14 (values are averages)
1x	Dissolved Oxygen (mg/L)	1.24	0.35	0.56	0.55	0.69
	Turbidity(NTU)	82.35	134.00	103.00	105.00	87.87
1.5x	Dissolved Oxygen (mg/L)	0.62	0.55	0.58	0.52	0.79
	Turbidity(NTU)	81.00	105.50	129.00	98.75	111.80
2x	Dissolved Oxygen (mg/L)	0.62	0.56	0.54	0.50	0.57
	Turbidity (NTU)	88.30	130.50	112.00	111.50	108.66
2.5x	Dissolved Oxygen (mg/L)	0.64	0.52	0.61	0.48	0.68
	Turbidity (NTU)	85.50	125.50	116.00	79.10	102.50
	Day 14 Results					
	Dissolved Oxygen (mg/L)	Turbidity (NTU)				
1x replicate 1	0.58	105.00				
1x replicate 2	0.64	87.60				
1x replicate 3	0.85	101.00				
Average	0.69	97.87				
1.5x replicate 1	0.89	115.50				
1.5x replicate 2	0.78	119.00				
1.5x replicate 3	0.69	100.90				
Average	0.79	111.80				
2x replicate 1	0.57	106.50				
2x replicate 2	0.55	107.50				
2x replicate 3	0.60	112.00				
Average	0.57	108.67				
2.5x replicate 1	0.60	109.50				
2.5x replicate 2	0.72	105.00				
2.5x replicate 3	0.72	93.00				
Average	0.68	102.50				